DECLARATION OF ALFRED C. NICHOLS, PH.D.

My name is Alfred C. Nichols. I am over 18 years of age and I currently reside in Jacksonville, Alabama. I have personal knowledge of the facts set forth in this declaration.

2. I am presently employed by Jacksonville State University in Jacksonville, Alabama as an Associate Professor of Chemistry.

3. After receiving my Ph.D., I worked at the University of Texas Medical Branch ("UTMB") in Galveston, Texas, where I began studying a specific set of excitatory amino acid neurotransmitter receptors that selectively bind *N*-methyl-D-aspartic acid ("NMDA"). Over-stimulation of these "NMDA receptors" has been implicated in a number of central nervous system disorders. Accordingly, NMDA antagonists are believed to have therapeutic benefit as a result of neuroprotective and anticonvulsant properties.

4. The NMDA receptor sites comprise a subset of excitatory receptors that are activated by L-glutamic acid. The receptor complex also has a strychnine-insensitive binding site for glycine. For channel opening to occur, apparently both glutamate and glycine binding sites must be occupied. Consequently, antagonism of either glutamate or glycine binding inhibits NMDA receptors.

5. My research with NMDA receptors and antagonists led to my work with kynurenic acid derivatives. Kynurenic acid derivatives have been shown to be a competitive inhibitor of glycine binding at the NMDA receptor.

6. My research with kynurenic acid derivatives led to the syntheses of novel 4-hydroxyquinaldic acid derivatives for use as photoaffinity probes for NMDA receptors, which became the subject of U.S. Patent No. 5,028,707 issued in 1991 and U.S. Patent No. 5,344,922 issued in 1994.

7. Continuing research with kynurenic acid derivatives led to the syntheses of novel 4-amino substituted derivatives for use as NMDA antagonists, such as 4-methylamino-5,7-dichloro-2-quinoline carboxylate, which became the subject of U.S. Patent No. 5,493,027 issued in 1996.

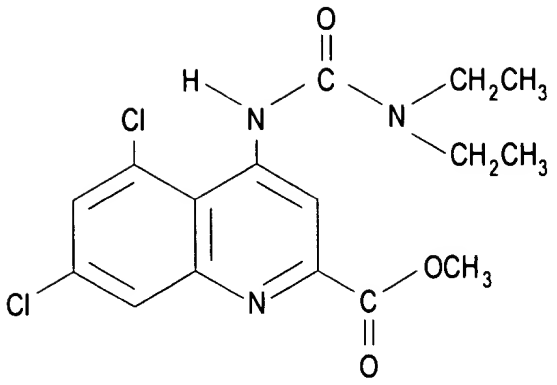
8. On or about February 15, 1994, I conceived of a synthesis method of forming a 4-urea derivative of a 4-amino-2-carboxyquinoline compound. I decided that phosgene [$COCl_2$] may be reactive enough to attach its acyl carbon [C] to the 4-amino group of the 4-amino-2-carboxyquinoline compound, after which, a secondary amine [N] could be attached to the carbonyl group [CO], thereby forming a 4-urea group. I decided that a di-substituted

secondary amine was preferable because (1) it would more likely attach to the carbonyl group [CO] because it was a stronger Lewis base and (2) there was not a risk of it forming a dimer with another quinoline structure. I could not find phosgene [$COCl_2$] commercially available, but was able to find triphosgene [$CO(OCCl_3)_2$], which was an acceptable alternative to phosgene. I decided to use diethylamine [$NH(ethyl)_2$] as the di-substituted amine.

9. On March 23, 1994, I began the first experiment according to the new synthesis method, wherein I first reacted triphosgene with 4-amino-7-chloro-2-carboxyquinoline methyl ester to attach triphosgene's carbonyl group [CO] to the 4-amino group and then reacted diethylamine to attach the secondary amine [N] to the carbonyl group [CO]. I recorded this experiment on page 94A-43 in my Lab Book (Nichols Exhibit 2020), which also documents the expected product having a 4-diethyl urea substitution ((N,N-diethyl)-4-ureido-7-chloro-2-carboxyquinoline methyl ester). However, I was unable to successfully isolate the expected product.

10. On April 11, 1994, I began another synthesis wherein I first reacted triphosgene with 4-amino-5,7-dichloro-2-carboxyquinoline methyl ester to attach triphosgene's carbonyl group [CO] to the 4-amino group and then reacted diethylamine to attach the secondary amine [N] to the carbonyl group [CO]. I recorded this experiment on pages 94A-63 and 94A-64 in my Lab Book (Nichols Exhibit 2030), which also documents the expected product having a 4-diethyl urea substitution ((N,N-diethyl)-4-ureido-5,7-dichloro-2-carboxyquinoline methyl ester) on page 94A-63.

11. I labeled a sample from the April 11, 1994 experiment 94A-64-II. The labels applied to my samples correspond to particular samples from particular pages from my Lab Books. For example, sample 94A-64-II corresponds to sample II from page 64 of my Lab Book No. 94A. I had a NMR spectrum performed on sample 94A-64-II. NMR spectra are used to identify chemical structures. A spectrum data sheet was generated from the NMR spectrum of sample 94A-64-II (Nichols Exhibit 2031), which indicated that the expected product having a 4-diethyl urea substitution ((N,N-diethyl)-4-ureido-5,7-dichloro-2-carboxyquinoline methyl ester) was successfully produced. The NMR spectrum data sheet does not indicate the date that the NMR spectrum was performed; however, page 94A-64 of my Lab Book (Nichols Exhibit 2030) includes an entry dated April 28, 1994 relative to the NMR spectrum wherein I note the apparent success of the synthesis ("looks like product is there!"). The structure of (N,N-diethyl)-4-ureido-5,7-dichloro-2-carboxyquinoline methyl ester is:

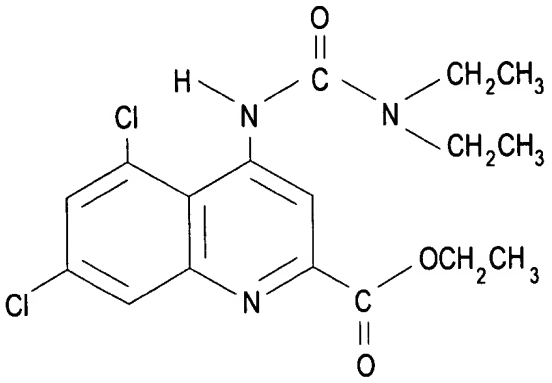


12. On May 3, 1994, I began a synthesis wherein I first reacted triphosgene with 4-tosylamino-5,7-dichloro-2-carboxyquinoline methyl ester to attach triphosgene's carbonyl group [CO] to the 4-amino group and then reacted diethylamine to attach the secondary amine [N] to the carbonyl group [CO]. I recorded this experiment on pages 94A-81, 94A-83 and 94A-85 of my Lab Book (Nichols Exhibit 2032), which also documents the expected product having a 4-diethyl urea substitution ((N,N-diethyl)-4-ureido-5,7-dichloro-2-carboxyquinoline methyl ester) on page 94A-83. The structure of (N,N-diethyl)-4-ureido-5,7-dichloro-2-carboxyquinoline methyl ester is shown above. I labeled a sample from this experiment 94A-85-I and sent a sample to UTMB's NMR facility for NMR testing.

13. On May 13, 1994, UTMB's NMR facility performed a NMR spectrum on sample 94A-85-I. A spectrum data sheet (Nichols Exhibit 2034) was generated from the NMR spectrum of sample 94A-85-I, which includes a drawing of the chemical structure of (N,N-diethyl)-4-ureido-5,7-dichloro-2-carboxyquinoline methyl ester that was subsequently added to the data sheet by me. The NMR test results are consistent with the chemical structure of (N,N-diethyl)-4-ureido-5,7-dichloro-2-carboxyquinoline methyl ester. Page 94A-85 of my Lab Book (Nichols Exhibit 2032) includes an entry dated May 13, 1994 relative to the NMR spectrum wherein I note the apparent success of the synthesis ("proton NMR hits!!").

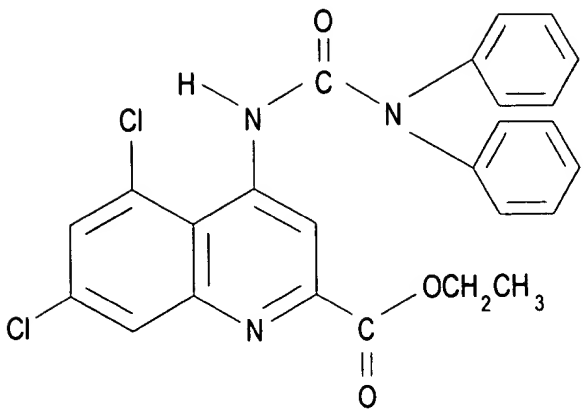
14. On July 1, 1994, I began a synthesis wherein I first reacted triphosgene with 4-tosylamino-5,7-dichloro-2-carboxyquinoline ethyl ester to attach triphosgene's carbonyl group [CO] to the 4-amino group and then reacted diethylamine to attach the secondary amine [N] to the carbonyl group [CO]. I recorded this experiment on pages 94B-20 and 94B-27 of my Lab Book (Nichols Exhibit 2022), which also documents the expected product having a 4-diethyl urea substitution ((N,N-diethyl)-4-ureido-5,7-dichloro-2-carboxyquinoline ethyl ester) on page 94B-20. I labeled a sample from this experiment

94B-27-I and sent 10 mg of 94B-27-I for elemental analysis. Elemental analyses are used to identify chemical structures. On July 22, 1994, I entered the results of the elemental analysis on page 94B-27 of my Lab Book (Nichols Exhibit 2022). The structure of (N,N-diethyl)-4-ureido-5,7-dichloro-2-carboxyquinoline ethyl ester is:



15. On July 22, 1994, after receiving the results of the elemental analysis for 94B-27-I, I sent 300 mg of sample 94B-27-I ((N,N-diethyl)-4-ureido-5,7-dichloro-2-carboxyquinoline ethyl ester) to NIH for anticonvulsant testing along with an Antiepileptic Drug Development (ADD) Registration Record (Nichols Exhibit 2037) showing the chemical structure of (N,N-diethyl)-4-ureido-5,7-dichloro-2-carboxyquinoline ethyl ester, its molecular weight, its molecular formula, and indicating that the compound had been identified by elemental analysis. The ADD Registration Record for sample 94B-27-I was processed by NIH on August 1, 1994, and assigned identification number ADD # 236001. Sample 94B-27-I (ADD # 236001) was tested on mice by NIH on August 20, 1994, and August 31, 1994. The August 31, 1994 test results (Nichols Exhibit 2023) indicated anticonvulsant activity of the compound. Specifically, the Threshold Tonic Extension (TTE) Test indicated protective activity at the 2-hour time interval.

16. On July 13, 1994, I began a synthesis wherein I first reacted triphosgene with 4-tosylamino-5,7-dichloro-2-carboxyquinoline ethyl ester to attach triphosgene's carbonyl group [CO] to the 4-amino group and then reacted diphenylamine to attach the secondary amine [N] to the carbonyl group [CO]. I recorded this experiment on pages 94B-25 and 94B-32 of my Lab Book (Nichols Exhibit 2024), which also documents the expected product having a 4-diphenyl urea substitution ((N,N-diphenyl)-4-ureido-5,7-dichloro-2-carboxyquinoline ethyl ester) on page 94B-25. I labeled a sample from this experiment 94B-32-III. The structure of (N,N-diphenyl)-4-ureido-5,7-dichloro-2-carboxyquinoline ethyl ester is:



17. I sent a sample of 94B-32-III for mass spectral analysis. On August 10, 1994, a fast atom bombardment (FAB) mass spectrum was performed in the Analytical Chemistry Center of the University of Texas Medical School in Houston on 94B-32-III. FAB mass spectra are used to identify chemical structures. A spectrum data sheet was generated from the FAB spectrum of 94B-32-III (page 2 of Nichols Exhibit 2039), which includes a drawing of the chemical structure of (N,N-diphenyl)-4-ureido-5,7-dichloro-2-carboxyquinoline ethyl ester that was subsequently added to the data sheet by me. The mass spectral test results are consistent with the chemical structure of (N,N-diphenyl)-4-ureido-5,7-dichloro-2-carboxyquinoline ethyl ester. Page 94B-32 of my Lab Book (Nichols Exhibit 2024) includes an entry dated August 12, 1994 relative to the mass spectrum wherein I note the apparent success of the synthesis ("got great mass spectrum").

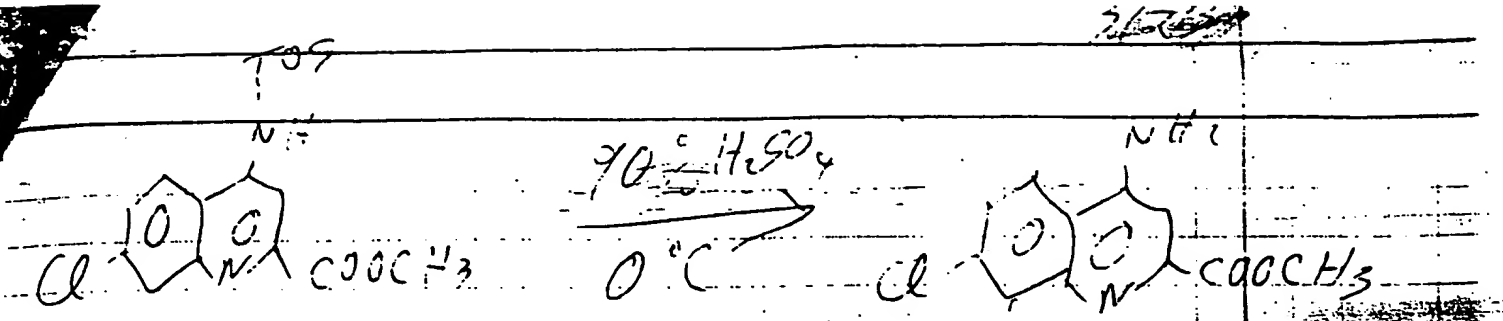
18. On August 12, 1994, after receiving results of the mass spectrum of sample 94B-32-III, I sent 280 mg of 94B-32-III ((N,N-diphenyl)-4-ureido-5,7-dichloro-2-carboxyquinoline ethyl ester) to NIH for anticonvulsant testing along with an Antiepileptic Drug Development (ADD) Registration Record (Nichols Exhibit 2040) showing the chemical structure of (N,N-diphenyl)-4-ureido-5,7-dichloro-2-carboxyquinoline ethyl ester, its molecular weight, its molecular formula, and indicating that the compound had been identified by mass spectrum. The ADD Registration Record for sample 94B-32-III was processed by NIH on August 30, 1994, and assigned identification number ADD # 236075. Sample 94B-32-III (ADD # 236075) was tested on mice by NIH on September 30, 1994, and October 4, 1994. The October 4, 1994 test results (page 3 of Nichols Exhibit 2025) indicated anticonvulsant activity of the compound. Specifically, the TTE Test indicated protective activity at the .25-hour and 2-hour time intervals.

19. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these

statements were made with the knowledge that willful false statements and the like made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent at issue in this interference.

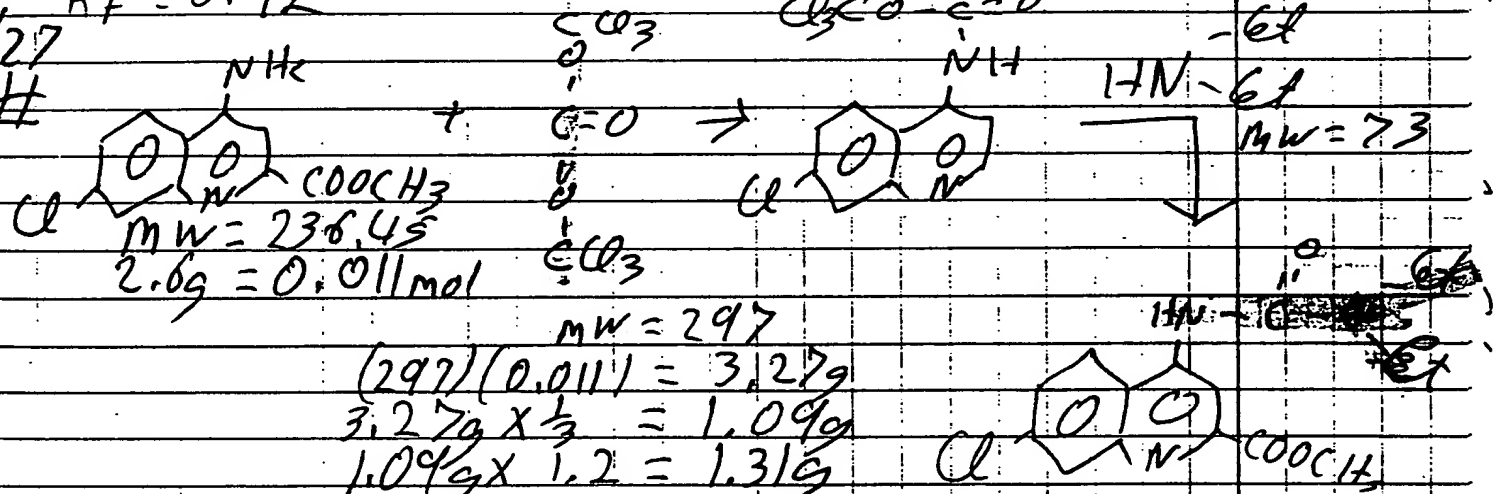
Executed on January 30, 2007.

Alfred C. Nichols

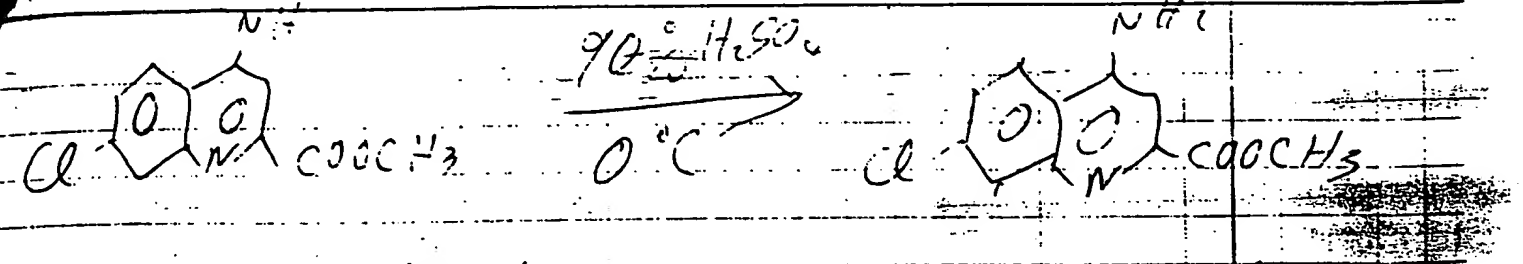


have 4g left from page 24; placed in 500 ml round bottom & cooled in ice water bath; also cooled 50 ml of 90% H_2SO_4 in ice water bath; added the acid to compound 2 & let stir at 0°C for 2 hr; poured over ice; collected ppt under vacuum; ran on TLC in DH: starting material gave fluorescent spot at solvent front; 43-I gave one spot at Rf = 0.92

127
#

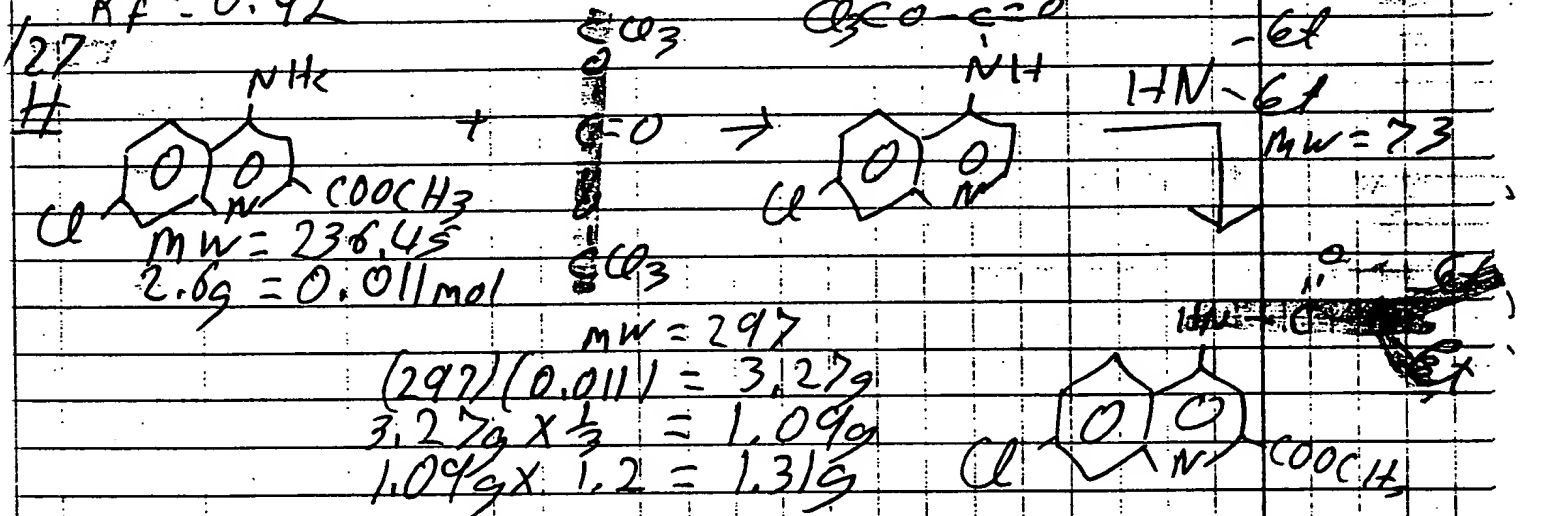


placed 2.6g of 43-I in 1l round bottom w 3 neck; cooled in ice water bath & added 50 ml anhydrous pyridine; added condenser & dropping funnel & covered w N_2; under good ~~weighed out~~ 1.3g triphosgene & added to pyridine soln; covered w N_2 & let stir at 0°C for 2 hr; ~~Then added~~ ~~dropwise 1.2 ml diethylamine dissolved in 20 ml~~ placed in N_2 gas then added dropwise 1.2 ml 20 ml anhydrous pyridine 30 min then at RT for 3 hr



have 4g left from page 24; placed in 500 ml round bottom & cooled in ice water bath; also cooled 50 ml of 90% H_2SO_4 in ice water bath; added 10 ml at a time to pyridine & let stir at 0°C for 2 hr; poured over ice & collected ten precipitate over vacuum; ran on TLC; DH: starting material gave fluorescent spot at solvent front; 43-I gave one spot at Rf = 0.92

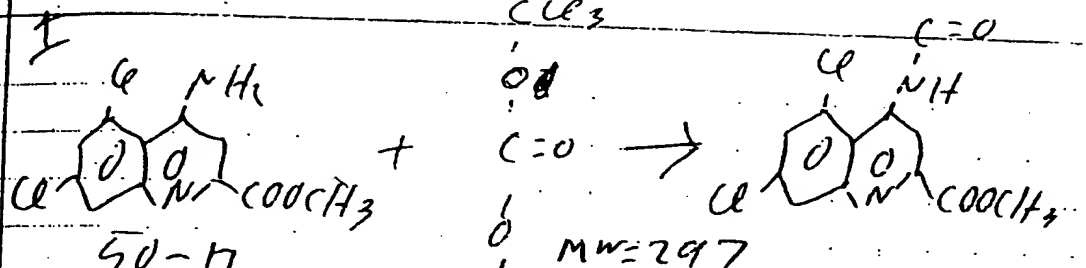
27



placed 2.6g of 43-I in 1l round bottom w 3 parts; cooled in ice water bath & added 50 ml anhydrous pyridine; added condenser & dropping funnel & covered w N_2; under hood weighed out 1.31g triphosgene & added to pyridine soln; covered w N_2 & let stir at 0°C for 2 hr; ~~Then added dropwise 1.2 ml diethyl amine dissolved in 20 ml~~ placed w N_2 gas then added dropwise 1.2 ml diethyl amine in 20 ml anhydrous pyridine; stirred at 0°C for 30 min then at RT for 30 min; poured onto ice and had

ee pool 99 4/11

I



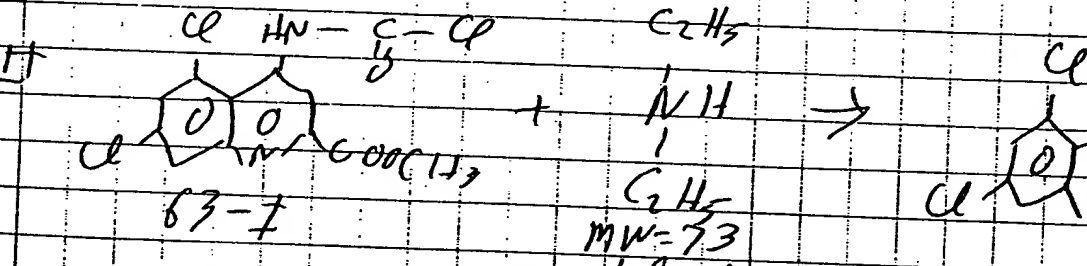
50-I
1g (0.0037 moles)

MW=297
CCl_3 (0.0074 mole)

cooled in ice water bath

in dry 250 round bottom w 2 parts placed 1g of 50-II & 10 ml of anhydrous pyridine, added condenser & tried to add 2.2 g triphosgene dropwise but this did not work well → it started reacting when pyridine was added then did not dissolve well w anhydrous THF; let warm up to room temp w stirring & continue to stir at r.t for 5 hrs

II



63-I

MW=73
use at least 0.54g
density = 0.719 g/ml
2g / 0.719 g/ml = 2.8ml

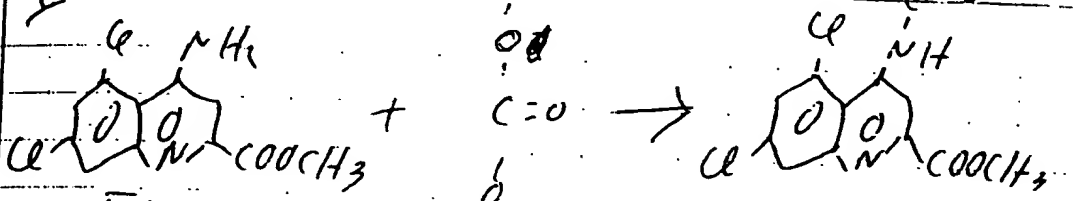
bubbled N_2 gas through reaction mixture then added 2.8 ml of diethylamine — this is more than enough to tie up unreacted phosgene; let stir at r.t for 1½ hrs & poured into ice/water shook out 3X w $CHCl_3$, combined & filtered over magnesium sulfate

4/12 ran on TLC in 5% MeOH; 50-II gave fluorescent spot at solvent front; 63-II gave spot at solvent front that does not fluoresce

evaporated off $CHCl_3$ under N2

see book 59 4/11

I



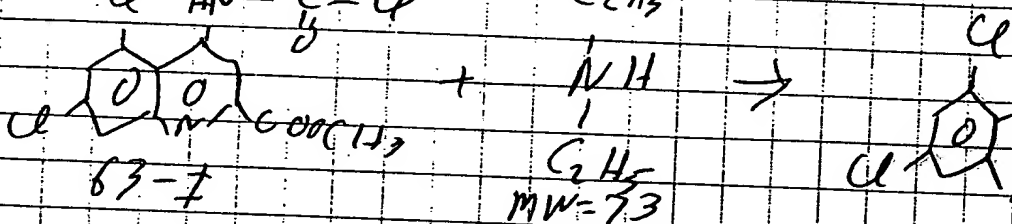
50-I
1g (0.0037 moles)

MW=297
CCl_3 (0.0074 mole)

cooled in ice water bath

in dry 250 round bottom w 2 necks placed 1g of 50-II & 10 ml of anhydrous pyridine, added condenser & tried to add 2.2 g triphosgene dropwise but this did not work well → it started reacting when pyridine was added then did not dissolve well w anhydrous THF; let warm up to room temp w stirring & continue to stir at r t for 5 hrs

II



63-I

MW=73
use at least 0.54g
density = 0.71 g/ml
2g / 0.71 g/ml = 2.8ml

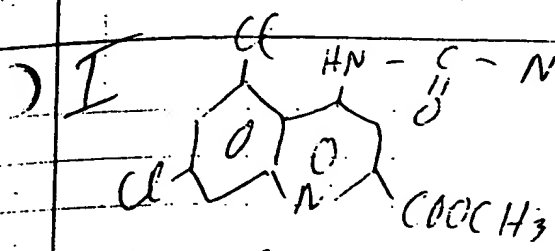
bubbled N_2 gas through reaction mixture then added 2.8 ml of diethylamine — this is more than enough to tie up unreacted phosgene; let stir at r t for 1½ hrs & poured into ice; shook out 3X w $CHCl_3$, combined & filtered over magnesium sulfate

4/12 ran on TLC in 5% MeOH; 50-II gave fluorescent spot at solvent front; 63-II gives spot at solvent front that does not fluoresce

evaporated off $CHCl_3$ under low

from page 63

4/12



63-II

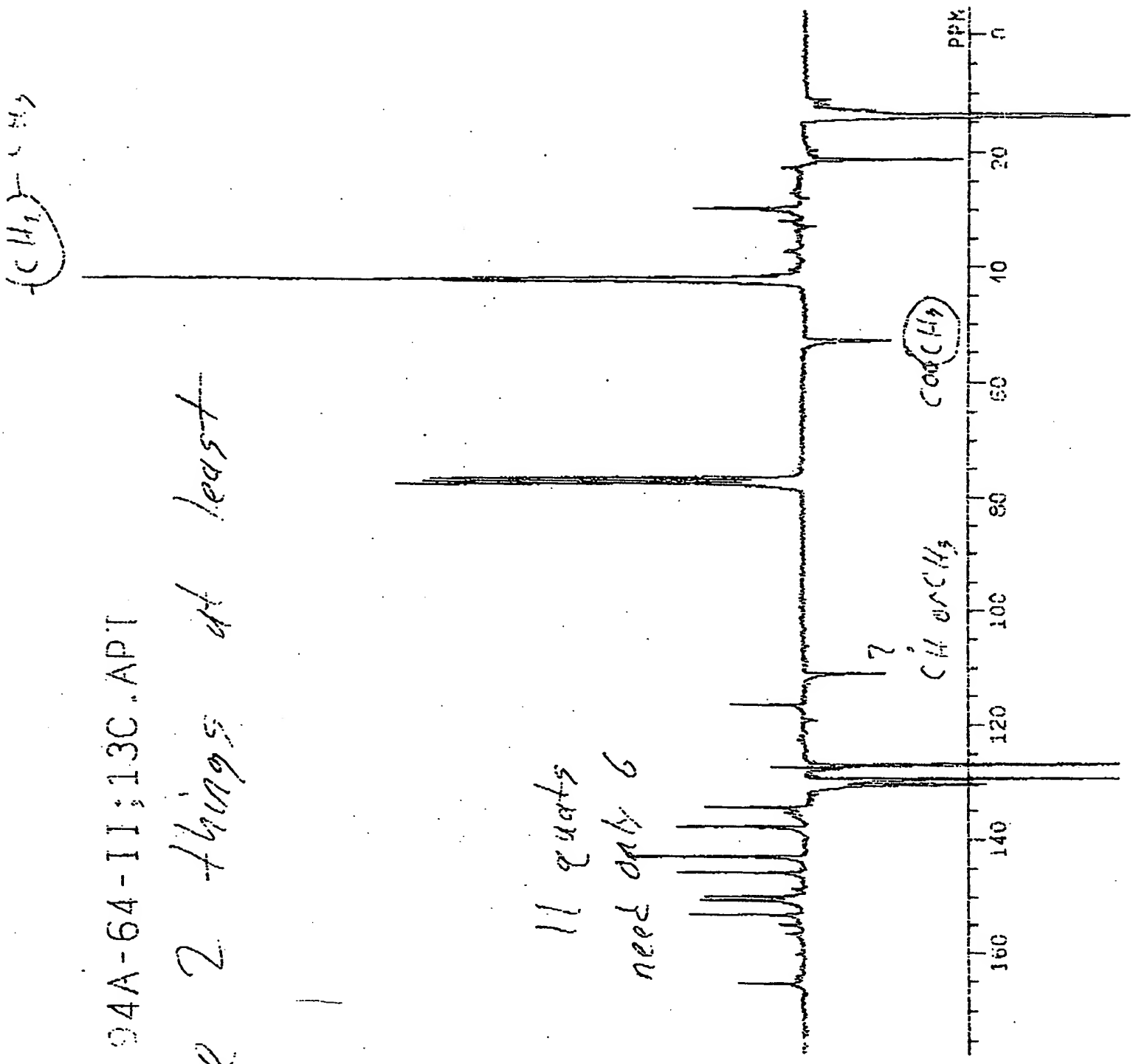
recovered dark oil from 63-II that smelled like pyridine, added ethylene chloride & shook out 2x w/ H_2O; filtered over magnesium sulfate & evaporated under hood to dark oil; added a little hexane — nothing dissolved, decanted hexane & added ethyl ether, most of it residue dissolved; filtered & added a little MeOH to filtrate, labeled as 64-II; placed under hood & hoped like hell something would crystallize — it didn't!; next dissolved in MeOH & filtered again & ran on TLC in 10% EtOH → spot at solvent front which is slightly fluorescent

4/28 — NMR; not pure — see too many aromatic carbons — but looks like product is there!

have $-CH_2-CH_3$, 3 C-H aromatic carbons, methoxy methyl

5/5 stirred w dilute NaOH — yellow color went into aqueous; filtered & made filtrate acid; got formation of yellow ~~ppt~~ precipitate; labeled as 64-III

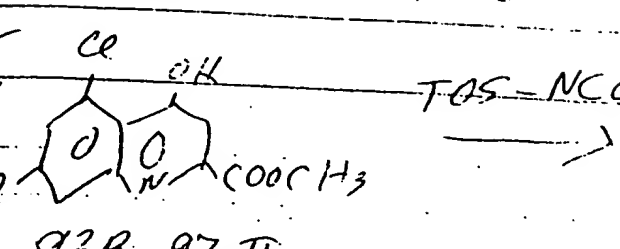
5/6 ran 64-III on TLC in 10% EtOH; got large spot at Rf ~ 0.45 which matched 63-I; also got two smaller spots below this



Nichols EXHIBIT 2031

acc 93B-100

I → TOS-NCC → 5 1/3

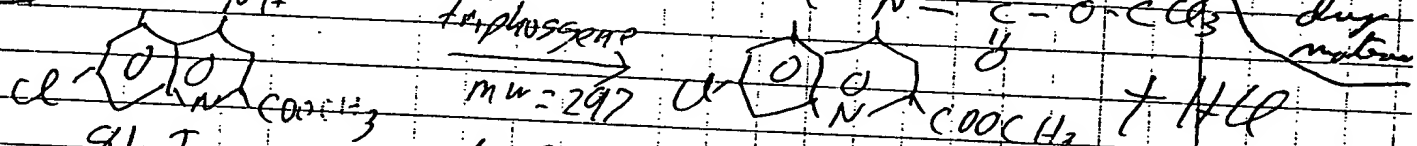


93B-97-II

5g

in dry 1L round bottom placed 5g of dry 93B-97-II, 200 ml ~~[illegible]~~ anhydrous acetonitrile & 7g p-toluenesulfonyl isocyanate, covered w N_2 added condenser & heated to reflux for 3h, let cool, collected yellow precipitate on vacuum, ran on TLC in 9:1 : 81-I spot at Rf = 0.88 that did not appear in 97-II spot at Rf = 0.7 ~~[illegible]~~ 6.5g dry material

II → triphosgene → mw = 297

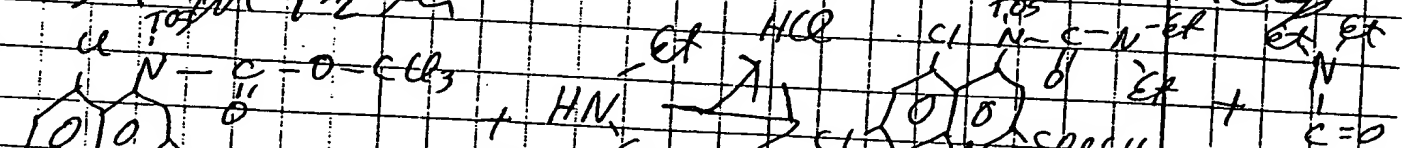


81-I
3.5g
MW = 425
(0.0082 moles)

use
~~2.5g = 3X~~
2.0

in dry 250 ml round bottom w 2 ports placed 3.5g of 81-I & cooled in ice water bath, added 2g (0.0067 moles → 0.02 moles phosgene or 2.5X) triphosgene & 10 ml THF (anhydrous), w stirring added 10 ml anhydrous pyridine dropwise & color of suspension changed from yellow to white then back to yellow, let warm to room temp & stir for 1½ hr

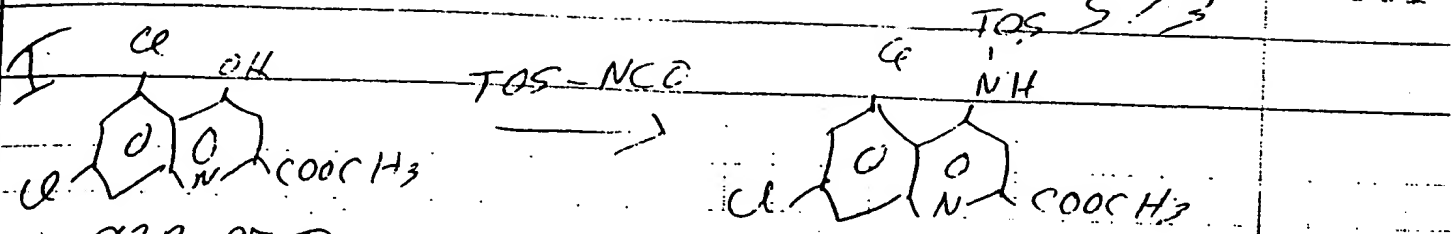
III → HCl



use
0.04 moles (mw = 73 → 2.9g → 4.1ml)

added 4.1 ml of diethyl to 81-II & let stir mixture turned dark brown

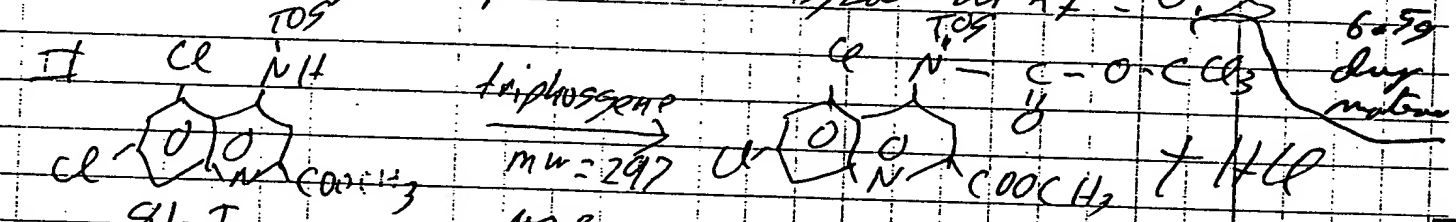
acc 93B-100



93B-97-II

5g

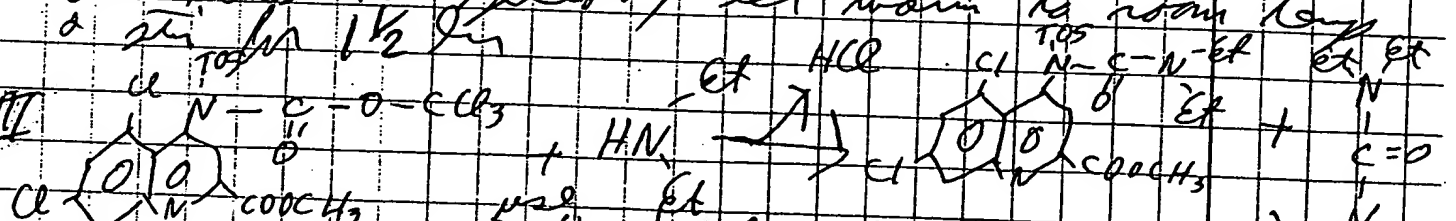
in dry 1L round bottom placed 5g of dry 93B-97-II, 200 ml anhydrous acetonitrile & 7g p-toluenesulfonyl isocyanate, covered w N_2 added condenser & heated to reflux for 3 hr, let cool, collected yellow precipitate on vacuum, ran on TLC in 1:1 : 8:1-I gave mat at Rf = 0.88 that did not fluoresce, 97-II gave fluorescent mat at Rf = 0.75 weighed 6.5g dry material



91-I
3.5g
MW-425
(0.0082 moles)

use
~~2.5g = 3X~~
2.0

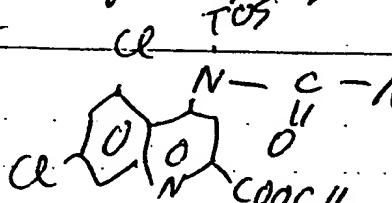
in dry 250 ml round bottom w 2 parts placed 3.5g of 91-I & cooled in ice water bath; added 2g (0.0067 moles → 0.02 moles phosgene or 2.5X) triphosgene & 10 ml THF (anhydrous); w stirring added 10 ml anhydrous pyridine dropwise, color of suspension changed from yellow to white then back to yellow, let warm to room temp & stir for 1½ hr



use
0.04 moles (MW = 73 → 2.9g → 4.1 ml)

added 4.1 ml of diethyl amine dropwise to 91-II & let stir at r.t. for 1 hr, mixture turned dark brown & poured over ice to

from page 81 5/4

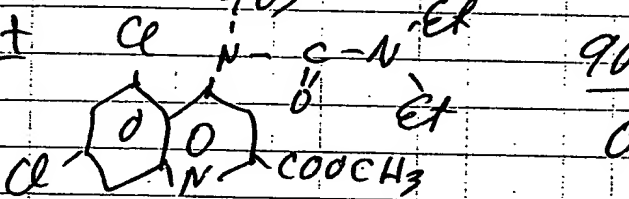


81-III

81-III

dried out 4X w $CHCl_3$, combined organic portions & filtered over magnesium sulfate; ran on TLC in OH: 81-III gave 2 spots — flourescent spot at solvent front & non-flourescent spot at Rf = 0.85 which matched 81-I — This looks good!

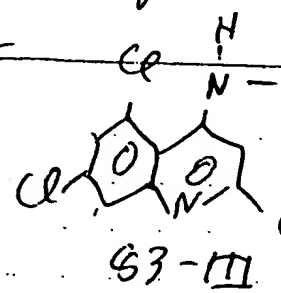
evaporated off $CHCl_3$ & recovered dark oil that smelled like pyridine; cooled in ice water bath



cooled 20 ml of 90% H_2SO_4 in ice water bath; w stirring slowly added the oil to 81-III; let stir at 0°C for 1 3/4 hrs; poured over ice; slowly raised pH w/ NaOH; at pH 0.75 got formation of small amt of orange goo; filtered out goo & labeled as 83-II — goo soluble in acetone; continued to raise pH of filtrate

515 @ raised pH to 6.3 — shook out 4X w ethyl acetate; dissolved 83-II in ethyl acetate (it had formed orange cystals as acetone evaporated); combined acetate portions & filtered over a little charcoal; labeled as 83-III; ran on TLC in OH: got large flourescent spot at solvent front & small spot at Rf = 0.40; (4-NH$_2$ also gives flourescent spot at solvent front — sol 93B-100); ran TLC in ethylene chloride + small spot at origin & flourescent spot at Rf = 0.69 [illegible] TLC

from page 83



83-III

5/5 reduced vol of 83-III & added a little NaOH → got formation of a red precipitate, collected over vacuum, washed w a little $CHCl_3$ & let air dry / labeled as 85-I

5/6 ran TLC on 85-I w/ OH → one spot at Rf = 0.45 that did not fluoresce

		expected	found	expected for sulfate salt
C = 16	= 192	= 51.89%	42.15	41.0%
H = 17	= 17	= 4.59	3.96	4.1
N = 3	= 42	= 11.35	8.45	9.0
O = 3	= 48	= 12.97		
Cl = 2	= 71	= 19.19		
	370	99.99		

5/13 proton NMR hits!!

ran mp = ? 260°C dec / ran TLC on analysis / somewhat soluble in $CHCl_3$

elemental analysis off but ratios are close

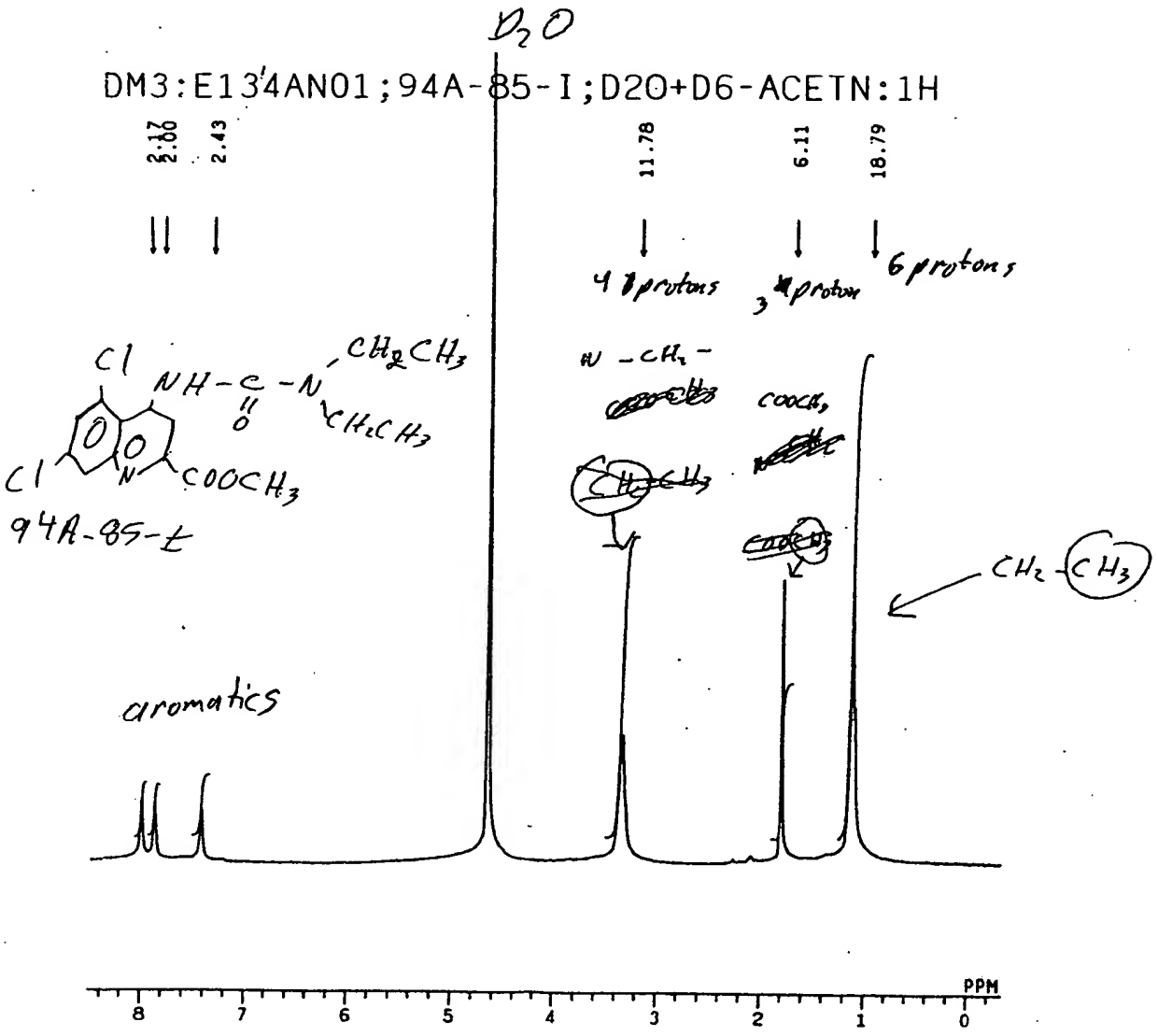
	expected	found
H/C	0.09	0.09
N/C	0.22	0.20
H/N	0.40	0.47

Need to purify & get NMR or mass spec

5/12 stirred 83-III w 6N HCl & filtered over a little Dowex / neutralized pH of filtrate w NaOH

5/13 proton NMR just — soluble in H_2O & not soluble in acetone — probably sulfate salt (MW = 468)

5/16 got good carbon NMR



94A-85-I

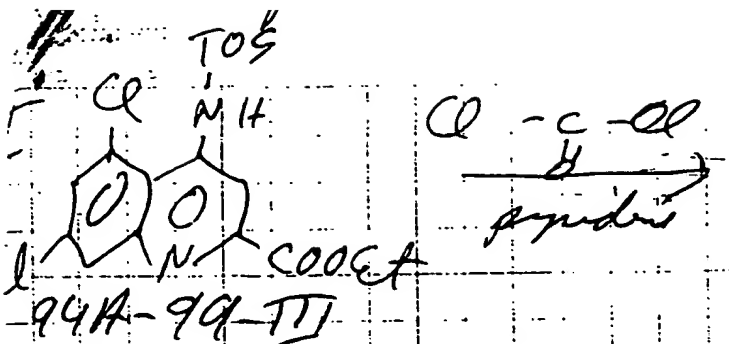
3-MAY-94 11:25:24

PEAK	7	
MXINT	723012600	
RESOL	0.3663575	Hz
RESOL	0.0013560	ppm
EXREF	2.0400000	ppm
OBS	-1767.08	Hz
ABOBS	270168.1000000	KHz
NGAIN	11	

COMNT DM3:E134AN01;94A-85-I;D2O+D6-ACETN:1H

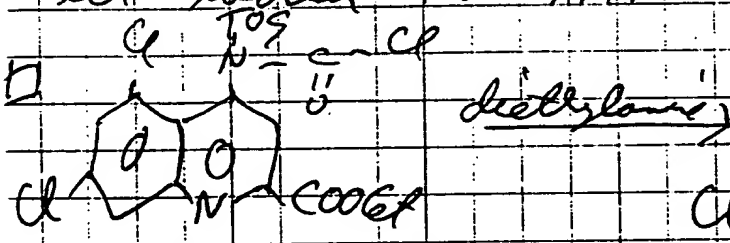
NO.	PPM	INT(%)	FREQ(Hz)	POSITION	BAR GRAPH
1	7.9917	7.50493	2159.08	7122	++
2	7.8629	7.05058	2124.28	7217	+
3	7.4140	6.11066	2003.01	7548	+
4	4.6531	100.00000	1257.11	9584	++++++++++++++++++++
5	3.3282	15.67389	899.18	10561	+++
6	1.8013	35.10741	486.66	11687	+++++++
7	1.1274	33.39196	304.58	12184	+++++++

Nichols EXHIBIT 2034

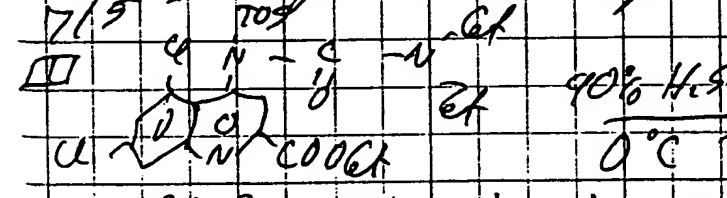


94A-99-III

in dry 250 ml round bottom w 2 ports placed 5g of dry 94A-99-III & added in ice water bath, added 2.99 triphosgene (excess), ~~added~~ covered w N_2 & added condenser & dropping funnel, dropwise w stirring added 50 ml anhydrous pyridine (do not have any THF); let warm to r.t. & stir for 1 hr,



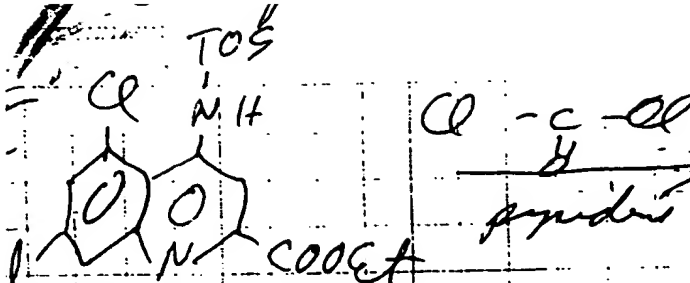
flushed 20-I w N_2, added 5 ml diethylamine dropwise, yellow soln turned dark brown, let stir at r.t. for 2 hr, poured over ice, collected orange precipitate over vacuum, washed w H_2O & let air dry, ran on TLC in 70% EtOAc/Hex, 20-II gave one spot at solvent front, 99-III gave one spot at R_f = 0.91



cooled 20-II in ice water bath, then cooled 20 ml 90% H_2SO_4, added acid soln to compound & let stir at 0°C for 2 hr, poured over ice & added a little NaOH, collected yellow precipitate over vacuum, ran on TLC w 70% EtOAc/Hex at solvent front, both II

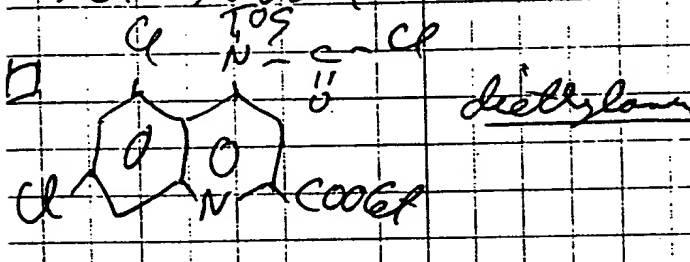
Nichols EXHIBIT 2022

Important: Place card unde

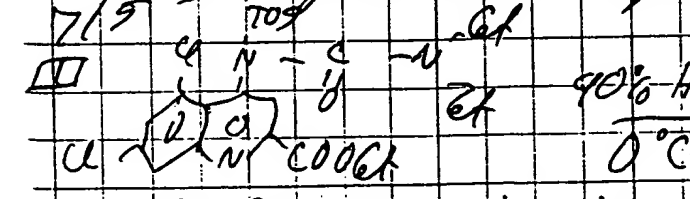


94A-99-III

In dry 250 ml round bottom w 2 port placed 5g of dry 94A-99-III & cooled in ice water bath, added 2.99 triphosgene (excess), ~~covered~~ covered w N_2 & added condenser & dropping funnel, dispersed w stirring, added 50 ml anhydrous pyridine (do not have any THF), let warm to r.t. & stir for 1 hr,



Refluxed 20-I w N_2, added 5 ml diethylamine dropwise, yellow soln turned dark brown, let stir at r.t. for 2 hr, poured over ice, collected orange precipitate per vacuum, washed w H_2O & let air dry, ran on TLC in 20-II 99-III gave one spot at solvent front, one spot at R_f = 0.91



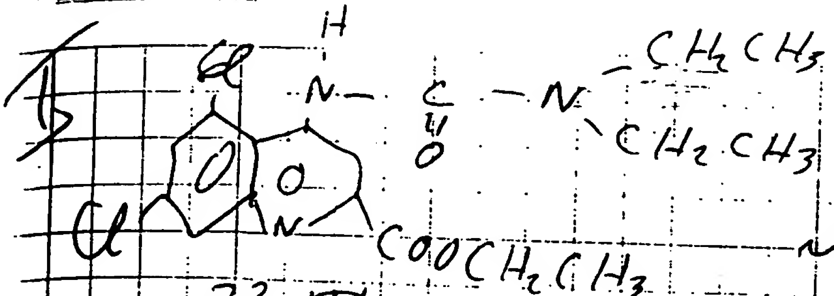
Cooled 20-II in ice water bath, also cooled 20 ml 90% H_2SO_4, added acid soln to compound & let stir at 0°C for 2 hr, poured over ice & added a little NaOH, collected yellow precipitate per vacuum, ran on TLC w 20-II one spot at solvent front, both II & III are soluble in CHCl

did reaction 90%

Important: Place card under blue copy

Name: from Roger's   7/15/94
Experiment:

027



Dried 23-III
need to see if it is salt

23-III

Wrong

				expected	corrected	found	+1 H_2O
C =	17	=	204	~~55.43%~~	53.13	51.74	~~52.85~~
H =	19	=	19	~~5.16~~	4.95	4.91	~~5.44~~
N =	3	=	42	~~11.41~~	10.94	10.48	~~10.88~~
~~O =~~	~~3~~	~~=~~	~~32~~	~~8.70~~	12.50		
Cl =	2	=	71	~~19.29~~	18.49		
			368	99.99%			

computes for $N_3C_{17}H_{20}$

ran MP = 90°C
sent 10 mg for analysis

~~for 1/2 H_2SO_4~~ expected
~~C = 48.92~~
~~H = 4.80~~
~~N = 10.07~~

~~for 1 H_2O~~
~~C = +2.15%~~
~~H = +10.78%~~
~~N = +3.82~~

~~for 1/2 H_2SO_4~~
~~C = −5.45%~~
~~H = −2.24~~
~~N = −3.91~~

9/26 active in TTE ~~test~~ at 100 mg/kg

7/22 for 1 H_2O
C = 50.75
H = 5.22
N = 10.45
O = 15.92
Cl = 17.66
100.00

for 1/2 H_2SO_4
C = 47.11
H = 4.62
N = 9.70

↖ this looks like +1 H_2O

$C_{17}H_{21}N_3O_4Cl_2$
MW = 402

sent 300 mg to NIH
sent 100 mg to Larry Snell
NIH # 238001

8/1/94

NATIONAL INSTITUTES OF HEALTH

ADD REGISTRATION RECORD

mplete one (1) form (both sides) for each compound.
plicate information need not be repeated.

v-p 419

ADD # 00236000

NAME OF SUPPLYING ORGANIZATION: University of Texas Medical Branch

DIRECT CORRESPONDENCE TO:

LAST NAME	FIRST NAME	INITIAL	DEGREE
Nichols	Al	C	Ph.D.

STREET ADDRESS: Pharmacology J-31

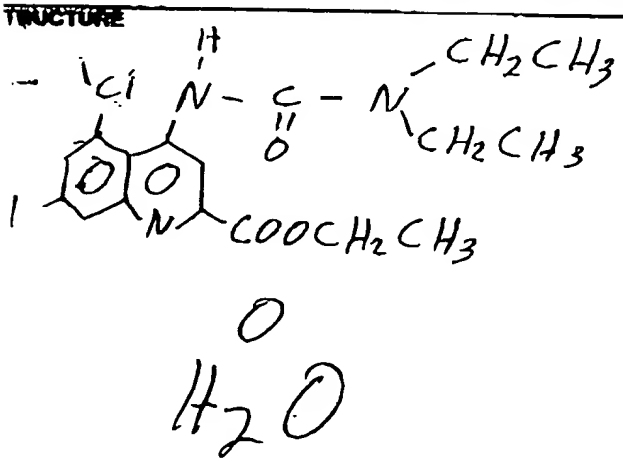
CITY	STATE	ZIP CODE
Galveston	TX	77555

TELEPHONE (Area Code): 409 | 772 | 9659

COMPOUND IDENTIFICATION: 94B-27-1

CHEMICAL NAME (If Known):

STRUCTURE



H_2O

MOLECULAR WEIGHT: 402

MOLECULAR FORMULA

C	H	N	O	CA	P	CL	S
17	21	03	04			02	

NA	F	MG	K

MELTING POINT (°C): 90

BOILING POINT (°C):

DECOMPOSITION
☐ YES ☒ NO

DATE PURITY WAS LAST ASCERTAINED: 7 | 20 | 94

BY WHAT METHOD
☒ CNH ANALYSIS
☐ OTHER (Specify)

DATE COMPOUND SHIPPED TO NINDS: 7 | 22 | 94

NUMBER OF CONTAINERS SHIPPED: 1

WEIGHT OF COMPOUND SHIPPED (mg.): 300

IF COMPOUND IS A DUPLICATE OF PREVIOUSLY TESTED COMPOUND

☒ PLEASE RETURN TO SUPPLIER

☐ IT IS NOT NECESSARY TO RETURN COMPOUND TO SUPPLIER. COMPOUND MAY BE USED AT NINDS DISCRETION FOR ANTI-CONVULSANT TESTING IN ANIMALS ONLY.

13

236001

A. STATE OF DEVELOPMENT

PATENTED ☐ YES (If yes, indicate proposed use in B block) ☐ NO

NUMBER | YEAR

IND ☐ YES (If yes, indicate proposed use in B block.) ☐ NO

DATE FILED:

KNOWN ACTIONS IN MAN — IF ANY (Indicate in B block.)

MARKETED ☐ YES (If yes, indicate approved use in B block.) ☐ NO

YEAR NDA APPROVAL

B. STATE OF DEVELOPMENT (Continued)

KNOWN ACTIONS	IND	NDA	PROPOSED OR APPROVED USE IN MAN
☐	☐	☐	ANTIEPILEPTIC
☐	☐	☐	NEUROLOGIC OTHER THAN ANTI-EPILEPTIC SEDATIVE — HYPNOTIC
☐	☐	☐	TRANQUILIZER
☐	☐	☐	MUSCLE RELAXANT
☐	☐	☐	STIMULANT, MOOD ELEVATOR
☐	☐	☐	ANALGESIC
☐	☐	☐	ANTICHOLINERGIC
☐	☐	☐	OTHER
☐	☐	☐	OTHER SYSTEM (Non CNS) (Specify in COMMENTS)

SPECIAL HANDLING INSTRUCTIONS
(Check all appropriate boxes.)

- ☐ UNSTABLE; COMPOUND CAN BE EXPECTED TO REMAIN STABLE FOR
- ☐ KEEP AWAY FROM HEAT
- ☐ KEEP AWAY FROM COLD
- ☐ DO NOT EXPOSE TO LIGHT
- ☐ INVESTIGATIVE DRUG, NOT FOR HUMAN USE
- ☐ OTHER

WE CAN SUPPLY ADDITONAL SAMPLES OF COMPOUND
(Check appropriate box.)

	500-1,000 mg	>1,000 mg
IMMEDIATELY UPON REQUEST	☐	☐
WITHIN 4 WEEKS	☐	☐
WITHIN 12 WEEKS	☒	☒
NOT AT ALL	☐	☐

KNOWN DATA — ANIMALS	ROUTE	SPECIES	TIME OF EFFECT	REFERENCE
LD_{50} mg/kg				
LD_{50} mg/kg				
MAXIMAL ELECTROSHOCK ED_{50} mg/kg				
OTHER ANTICONVULSANT TEST ED_{50}				
TOXICITY — KIND, DOSE				
TOXICITY — KIND, DOSE				
OTHER — SPECIFY EFFECT, DOSE				
OTHER — SPECIFY EFFECT, DOSE				
OTHER — SPECIFY EFFECT, DOSE				

COMMENTS

Could you please supply more submission forms

ANTICONVULSANT SCREENING PROJECT TEST RESULTS

THRESHOLD TONIC EXTENSION (TTE) TEST: Mice, i.p.

ADD # 236001 Supplier Code: 419 Date: 31-Aug-94

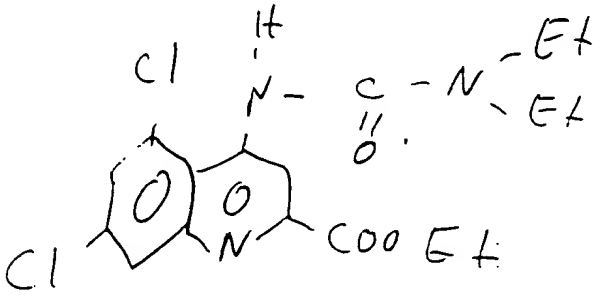
Solvent: MC (M&P, SB)

Reference: 266:2 Animal Weight: 21.0 to 25.0 g

Dose (mg/kg)	# Protected/# Tested .25 hr	.5 hr	1 hr	2 hr	4 hr	6 hr	8 hr
100	0/4	0/4	0/4	1/4	0/4	__/__	__/__
____	__/__	__/__	__/__	__/__	__/__	__/__	__/__

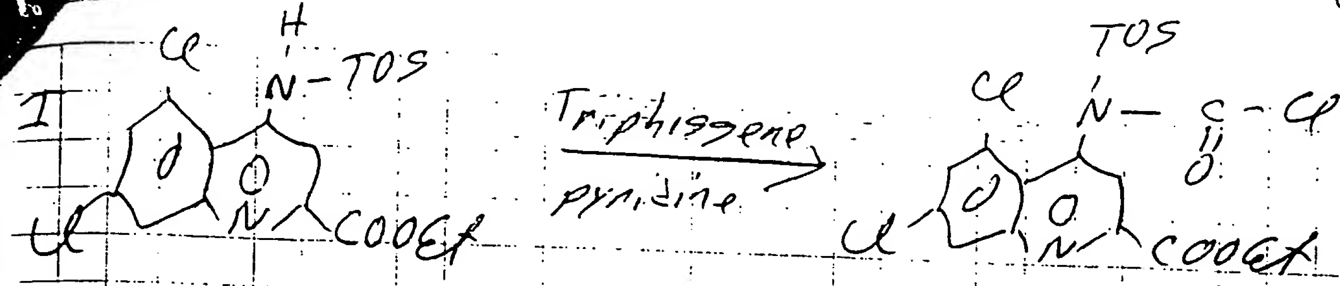
MES Confirmation

Dose (mg/kg)	# Protected/# Tested .25 hr	.5 hr	1 hr	2 hr	4 hr	6 hr	8 hr
____	__/__	__/__	__/__	__/__	__/__	__/__	__/__

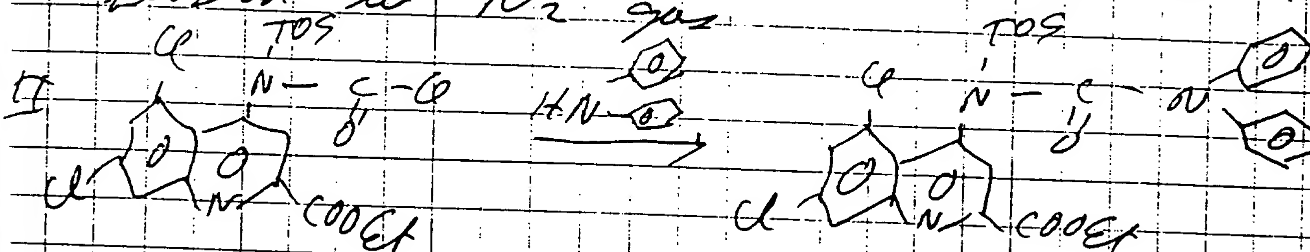


94B-27-1

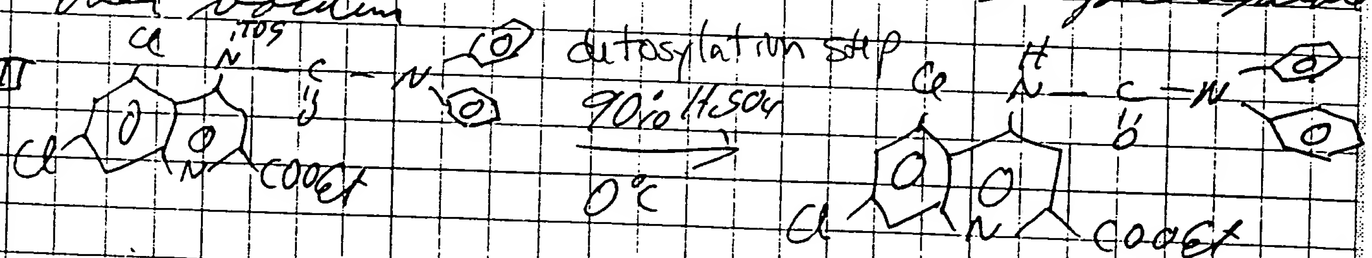
Nichols EXHIBIT 2023



in dry 250 ml round bottom w. 2 necks placed 5g of 94A-99-II; cooled in ice water bath; added 2.9g triphosgene, covered w N_2 & added condensor & dropping funnel; added dropwise 50 ml anhydrous pyridine w stirring; had evolution of gas; let warm to r.t. & stir for 1 hr; bubbled w N_2 gas



dropwise added 8.2g of diphenylamine dissolved in 40 ml of pyridine to 25-I; got great crimson color; let stir at r.t. for 2 hrs; poured over a lot of ice; collected orange precipitate over vacuum

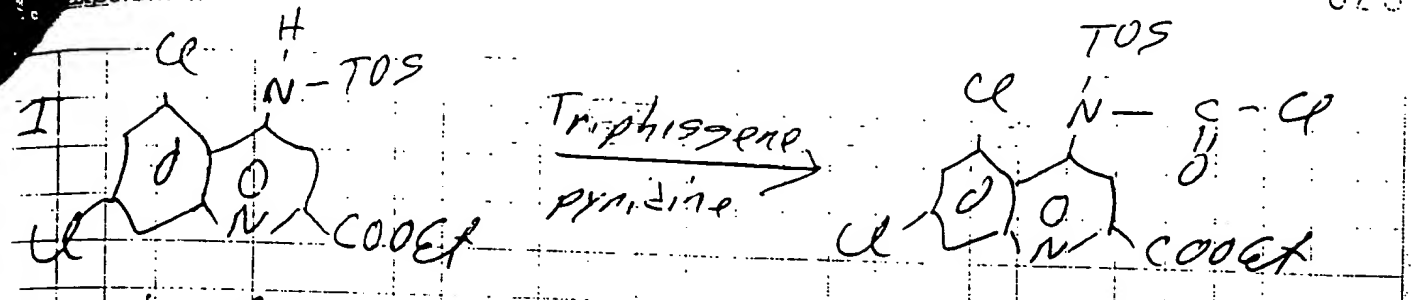


stirred 25-II in ice water bath; added 30 ml of 90% H_2SO_4; let stir at 0°C for 2 hrs; poured over ice; collected gummy precipitate over vacuum

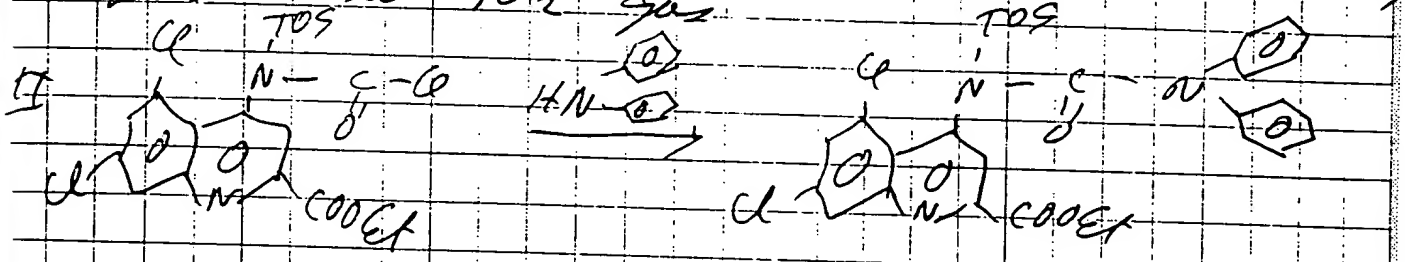
7/14 ran on TLC in ethylene chloride; diphenyl amine gives one spot at Rf 0.94; III [illegible] gives small spot here & chloride wash of III gives [illegible] spot at 0.9[illegible]

Important: [illegible]

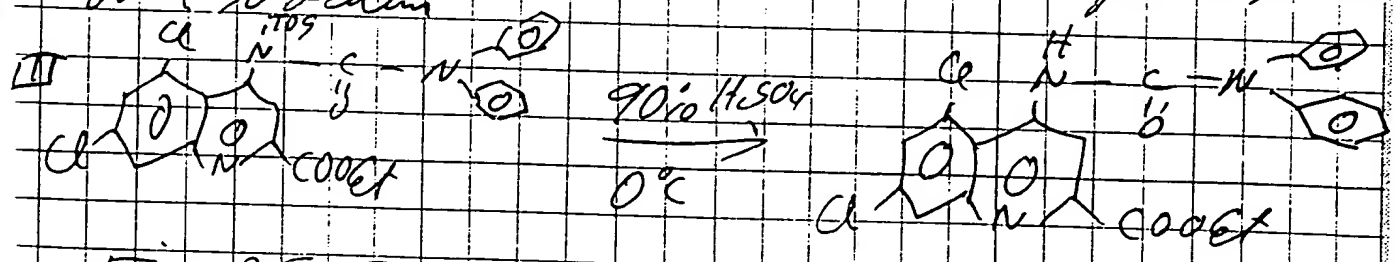
2/15/94



in dry 250 ml round bottom w 2 parts placed 5g of 944-99-II; cooled in ice water bath; added 2.99 triphosgene, covered w N_2 & added condenser & dropping funnel; added dropwise 50 ml anhydrous pyridine w stirring; had evolution of gas; let warm to r.t. & stir for 1 hr; blanked w N_2 gas.

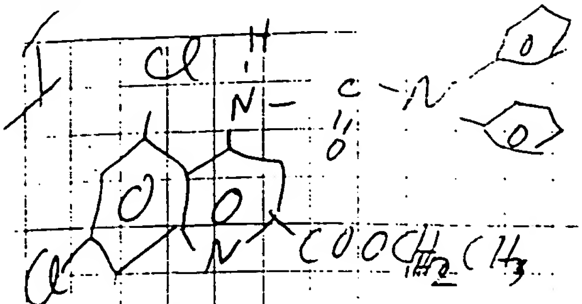


dropwise added 6.2g of diphenylamine dissolved in 40 ml of pyridine to 25-I; got great crimson color; let stir at r.t. for 2 hrs; poured over 9 lbs of ice; collected orange precipitate over vacuum



stirred 25-II in ice water bath; cooled 30 ml of 90% H_2SO_4; let stir at 0°C for 2 hrs; poured over ice; collected gummy precipitate over vacuum

7/14 ran on TLC in ethylene chloride; diphenyl amine gave one spot at Rf 0.94; III residue gives small spot here & spot at origin; ethylene chloride wash of III gave large spot at origin & large spot at 0.94 Important:



26-II

dissolved 26-II in ethyl acetate w a little heating, reduced vol by about 1/3 & added & equal vol of hexane; let sit overnight

7/26 added a little more hexane & collected white precipitate over vacuum; ran on TLC in ethylene chloride: precipitate gave one discrete spot at Rf = 0.31; the filtrate gave a streak to 0.31

ran mp = 195°C sharp

7/29 took some remaining 26-II & stirred in 6N HCl & filtered; labeled the undissolved residue as 32-II; added ice & NaOH to the filtrate & labeled it as 32-III

8/1 made 32-III basic in NaOH but nothing fell out;

ran TLC on 32-I & 32-II → each gives an spot at Rf - 0.23 in ethylene chloride

8/4 have NMR that seems to fit; product decomposes when heated to 80°C in DMSO — on NMR looks like hydrolysis giving off diphenyl amine & forming 'N-COOH'

C = 25 = 300
H = 19 = 19
N = 3 = 42
O = 3 = 48
409
Cl = 2 = 71
480

8/12 mass spectrum! 479 & 481

got great mass spectrum; sent 290 mg to NIH for testing
10 mg to Larry Snell
NIH # 236075

NIH active in TLC [illegible]

Important: Place card under blue copy

BEST AVAILABLE COPY

THE UNIVERSITY OF TEXAS
HOUSTON
HEALTH SCIENCE CENTER

Medical School
Analytical Chemistry Center

FAX TRANSMITTAL SHEET

DATE: August 11, 1994

TO: Dr. Al Nichols
Department of Pharmacology
UTMB-Galveston

FAX NUMBER: (409) 772-9642

FROM: William E. Seifert, Jr., Ph.D. Bill
Assistant Director
Analytical Chemistry Center
The University of Texas Medical School at Houston
6431 Fannin, Rm. 6.130 MSB
Houston, TX 77030

Telephone: (713) 792-5612
FAX: (713) 794-4226

Following is the FAB mass spectrum obtained from the analysis of your sample 94B-32-III. As you can see from the spectrum, the expected $[M+H]^+$ at m/z 480.1 was observed and with the expected isotope ratio for a compound containing two Cl atoms.

If you have any questions regarding these analyses, please do not hesitate to contact me.

TOTAL PAGES INCLUDING THIS SHEET: 5

THE UNIVERSITY OF TEXAS
HOUSTON
HEALTH SCIENCE CENTER

Medical School
Analytical Chemistry Center

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The University of Texas Medical School at Houston
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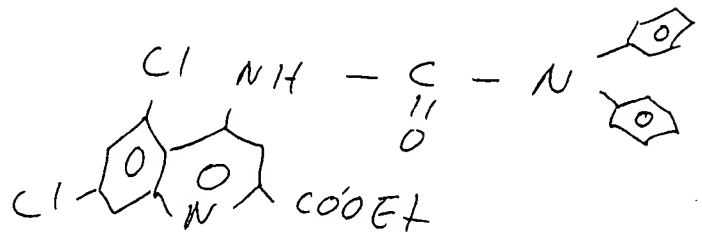
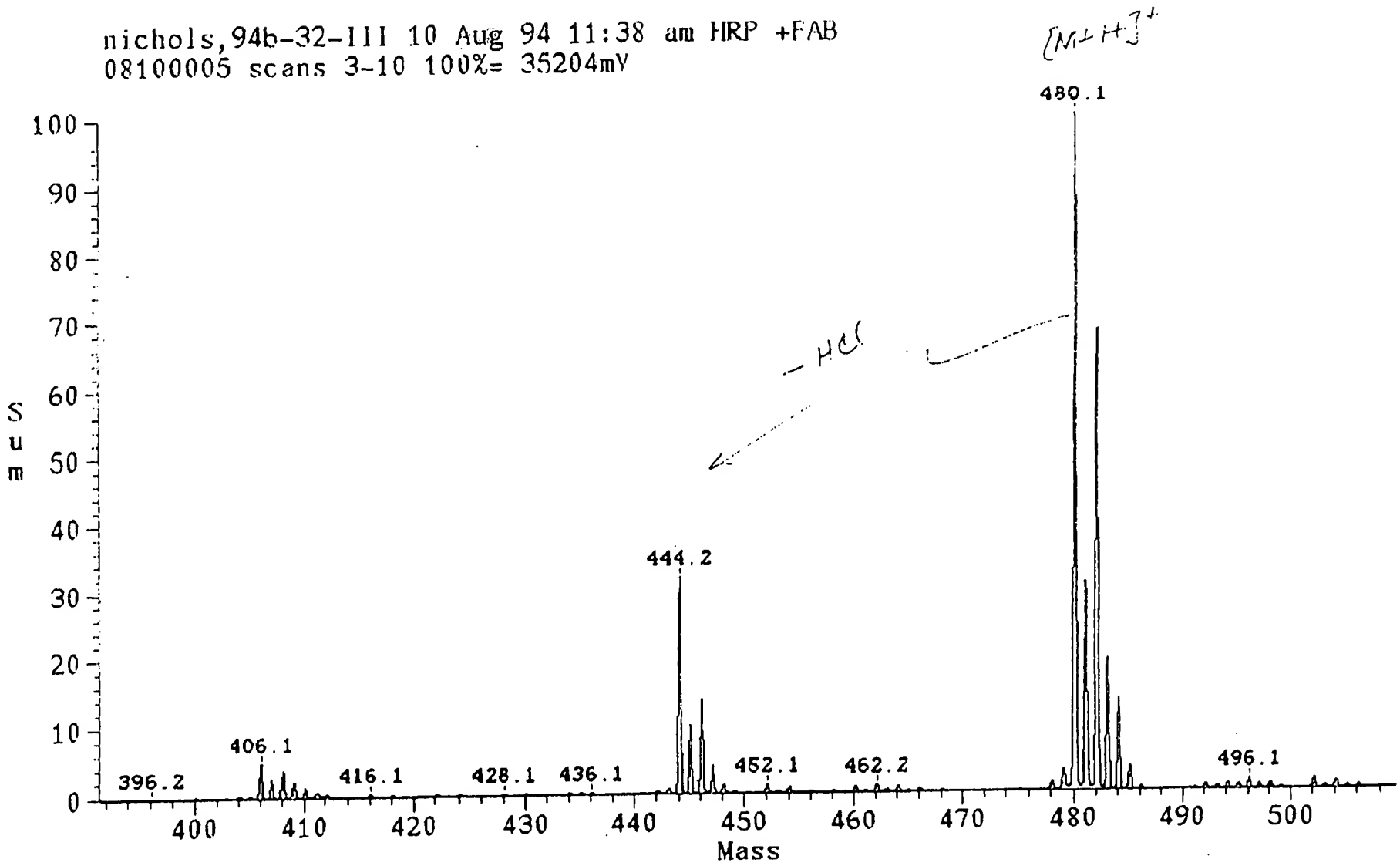
TOTAL PAGES INCLUDING THIS SHEET: 5


Cl
NH
C
O
N
Cl
O
N
COOEt


nichols,94b-32-III 10 Aug 94 11:38 am HRP +FAB
08100005 scans 3-10 100%= 35204mV
[M+H]+
480.1
-HCl
444.2
406.1
396.2
416.1
428.1
436.1
452.1
462.2
496.1
Sum
Mass
100
90
80
70
60
50
40
30
20
10
0
400
410
420
430
440
450
460
470
480
490
500

NATIONAL INSTITUTES OF HEALTH

ADD REGISTRATION RECORD

Complete one (1) form (both sides) for each compound. Duplicate information need not be repeated.

4/9 8/30/94

ADD # 0 0 2 3 6 0 7 9

NAME OF SUPPLYING ORGANIZATION: University of Texas Medical Branch

DIRECT CORRESPONDENCE TO:

LAST NAME	FIRST NAME	INITIAL	DEGREE
Nichols	A1	C	Ph.D.

STREET ADDRESS: Pharmacology J-31

CITY	STATE	ZIP CODE
Galveston	TX	77555

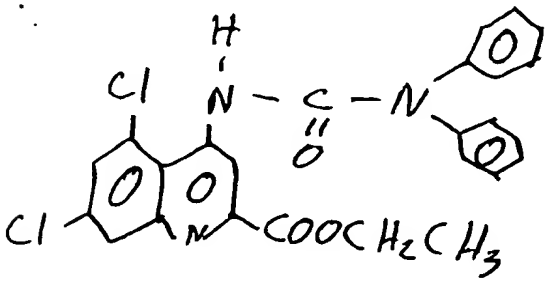
TELEPHONE (Area Code): 409 | 772 | 9659

COMPOUND IDENTIFICATION: 94B-32-H1

CHEMICAL NAME (If Known):

R-1780

STRUCTURE



MOLECULAR WEIGHT: 480

MOLECULAR FORMULA

C	H	N	O	CA	P	CL	S
25	19	03	03			02	

NA	F	MG	K

MELTING POINT (°C): 195

BOILING POINT (°C):

DECOMPOSITION: ☐ YES ☒ NO

DATE PURITY WAS LAST ASCERTAINED: 8 | 11 | 94

BY WHAT METHOD

☐ CNH ANALYSIS

☒ OTHER (Specify) mass spectrum

DATE COMPOUND SHIPPED TO NINDS: 8 | 12 | 94

NUMBER OF CONTAINERS SHIPPED: 1

WEIGHT OF COMPOUND SHIPPED (mg.): 280

IF COMPOUND IS A DUPLICATE OF PREVIOUSLY TESTED COMPOUND

☒ PLEASE RETURN TO SUPPLIER

☐ IT IS NOT NECESSARY TO RETURN COMPOUND TO SUPPLIER. COMPOUND MAY BE USED AT NINDS DISCRETION FOR ANTI- [redacted] ESTING IN ANIMALS ONLY.

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(Page 1)

Nichols EXHIBIT 2040

BEST AVAILABLE COPY

236075

A. STATE OF DEVELOPMENT

PATENTED ☐ YES (If yes, indicate proposed use in B block) ☐ NO

NUMBER | YEAR

IND ☐ YES (If yes, indicate proposed use in B block.) ☐ NO

DATE FILED:

KNOWN ACTIONS IN MAN — IF ANY (Indicate in B block.)

MARKETED ☐ YES (If yes, indicate approved use in B block.) ☐ NO

YEAR NDA APPROVAL

B. STATE OF DEVELOPMENT (Continued)

KNOWN ACTIONS	IND	NDA	PROPOSED OR APPROVED USE IN MAN
☐	☐	☐	ANTIEPILEPTIC
☐	☐	☐	NEUROLOGIC OTHER THAN ANTI-EPILEPTIC SEDATIVE—HYPNOTIC
☐	☐	☐	TRANQUILIZER
☐	☐	☐	MUSCLE RELAXANT
☐	☐	☐	STIMULANT, MOOD ELEVATOR
☐	☐	☐	ANALGESIC
☐	☐	☐	ANTICHOLINERGIC
☐	☐	☐	OTHER
☐	☐	☐	OTHER SYSTEM (Non CNS) (Specify in COMMENTS)

SPECIAL HANDLING INSTRUCTIONS (Check all appropriate boxes.)

- ☐ UNSTABLE; COMPOUND CAN BE EXPECTED TO REMAIN STABLE FOR
- ☒ KEEP AWAY FROM HEAT decomposes when heated in aqueous solution
- ☐ KEEP AWAY FROM COLD
- ☐ DO NOT EXPOSE TO LIGHT
- ☐ INVESTIGATIVE DRUG, NOT FOR HUMAN USE
- ☐ OTHER

WE CAN SUPPLY ADDITONAL SAMPLES OF COMPOUND (Check appropriate box.)

	500-1,000 mg	~1,000 mg
IMMEDIATELY UPON REQUEST	☐	☐
WITHIN 4 WEEKS	☒	☒
WITHIN 12 WEEKS	☐	☐
NOT AT ALL	☐	☐

KNOWN DATA—ANIMALS	ROUTE	SPECIES	TIME OF EFFECT	REFERENCE
LD_{50} mg/kg				
LD_{50} mg/kg				
MAXIMAL ELECTROSHOCK ED_{50} mg/kg				
OTHER ANTICONVULSANT TEST ED_{50}				
TOXICITY — KIND, DOSE				
TOXICITY — KIND, DOSE				
OTHER — SPECIFY EFFECT, DOSE				
OTHER — SPECIFY EFFECT, DOSE				
OTHER — SPECIFY EFFECT, DOSE				

COMMENTS

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
National Institutes of Health

National Institute of Neurological
Disorders and Stroke
Preclinical Pharmacology Section
Epilepsy Branch
7550 Wisconsin Avenue, MSC 9020
Federal Building, Room 114
Bethesda, Maryland 20892-9020
Phone Number: (301) 496-1846
FAX Number: (301) 496-9916

October 13, 1994

Dr. Al C. Nichols
Medical Branch
University of Texas
Pharmacology Building, J-31
Galveston, TX 77550

Dear Dr. Nichols:

Testing was recently completed for your compound ADD 236075. It was screened in both our standard identification screens as well as the new TTE test. The compound was not active in the standard screens but showed some protection at 1/4 and 2 hours in the TTE test. At this time this level of activity is not quite enough to qualify for additional tests. As more information is obtained from other TTE experiments we may change the criteria. If this occurs I will contact you possibly further considering some of your compounds.

In the meantime if you have any questions please feel free to contact me.

Sincerely yours,

James P. Stables
Assistant Chief
Preclinical Pharmacology Section
Epilepsy Branch
Division of Convulsive, Developmental
and Neuromuscular Disorders

ADD test data on
the ethyl ester compound

Nichols EXHIBIT 2025

--- IDENTIFICATION MICE I.P. ----------

Add ID:236075 Sponsor ID:419 Class:3

94B- 32-III

Solvent.....:MC Sol. Prep:M&P,SB
Date Started..:30-SEP-1994
Date Completed:30-SEP-1994
Reference.....:265:70
Animal Weight.:18.00 to 21.00 (g)
Test Comments.:

Time in Hours

TEST	DOSE mg/kg	FORM	0.50 #/F	CM	4.00 #/F	CM	0.25 #/F	CM	1.00 #/F	CM	2.00 #/F	CM	3.00 #/F	CM	6.00 #/F	CM	8.00 #/F	CM	# Dths
MES	30.00	SUS	0/1		0/1														
MES	100.0	SUS	0/3		0/3														
MES	300.0	SUS	0/1		0/1														
ScMET	30.00	SUS	0/1		0/1														
ScMET	100.0	SUS	0/1		0/1														
ScMET	300.0	SUS	0/1		0/1														
TOX	30.00	SUS	0/4		0/2														
TOX	100.0	SUS	0/8		0/4														
TOX	300.0	SUS	0/4		0/2														

94B-32-111

ANTICONVULSANT SCREENING PROJECT TEST RESULTS

THRESHOLD TONIC EXTENSION (TTE) TEST: Mice, i.p.

ADD # 236075 Supplier Code: 419 Date: 4-OCT-94

Solvent: MC (M&P,SB)

Reference: 266:72 Animal Weight: 21.0 to 25.5 g

Dose (mg/kg)	# Protected/# Tested .25 hr	.5 hr	1 hr	2 hr	4 hr	6 hr	8 hr
100	1/4	0/4	0/4	2/4	0/4	/__	/__
____	/__	/__	/__	/__	/__	/__	/__

MES Confirmation

Dose (mg/kg)	# Protected/# Tested .25 hr	.5 hr	1 hr	2 hr	4 hr	6 hr	8 hr
100	0/4	/__	/__	0/4	/__	/__	/__